Cancer Metabolism: Involvement in Oncogenesis and Cancer Development Provides New Therapeutic Targets

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Abstract

Metabolic regulation is an important new target in the battle against cancer. Through history, cancer metabolism has been underestimated. Metabolic research of cancer started promising with the discovery of aerobic glycolysis, known as the Warburg effect, but cancer studies mainly focused on other aspects. Only in recent years, research in cancer metabolism is rejuvenated and is considered hallmark in oncogenesis and cancer development. Cancer cells experience metabolic alterations which change energy production, biosynthesis and replicative behavior among others. Together, these cellular changes provide an altered physiological state which distinguishes them from heathy cells. Many oncogenes and tumor-suppressor genes are involved in the shifted metabolic regulation of cancer. Iconic cancer genes like Myc and p53 have great influence on regulatory pathways responsible for the metabolic shift. Lots of genes involved show completely different functions in cancer cells compared to normal cells; thereby provide new targets for drug development. The metabolic alterations include cellular changes necessary for metastasis. Regarding metastasis, metabolic regulators and environmental circumstances enable cells to leave their own original location and migrate to another tissue. Metastasizing cancer cells enhances the pathology and, eventually, contribute a great deal to cancer-induced mortality. In order to prevent cancer development, metastasis and the underlying pathology, promising research to develop metabolic interventions has been initiated. Metabolic interventions have the potential to become the new solution in the battle against cancer.

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Introduction

Cancer is a multifactorial group of diseases characterized by the uncontrolled growth and the spread of abnormal cells, also called metastasis (American Cancer Society, 2015). Cancer has been known throughout the entire recorded history. The first description of cancer was found in Egypt and dates from about 3000 BC (American Cancer Society, 2015). In that description of cancer was concluded: 'There is no treatment'. Even though cancer has been known for thousands of years, only research developments since the 19th century revealed the true causes and possible treatments for this complicated group of diseases. Cancer can be caused by internal irregularities, such as genetic mutations, hormone and immune conditions, and external factors, like tobacco smoking and infectious organisms (American Cancer Society, 2015). These factors cause cellular transformations involved in the growth process and cell-cell interactions which eventually can lead to the uncontrolled growth and metastasis. Metastasis of cancer cells is a significant cause of death. All cancers combined were the second biggest cause of death in the United States of America in 2014 (National Center for Health Statistics, 2016).

Cancer is a heterogenic group of diseases with lots of variations between cancer types. In attempt to create generality in cancer types, a lot of research has been done to determine which cellular transformations are key in developing cancer. These transformations are called the *hallmarks of cancer* (Hanahan, 2000). The hallmarks of cancer are generally accepted in science, but are an incomplete overview of all the similarities cancer types have. Increasing interest and research progression made Hanahan and Weinberg adapt the initial hallmarks of cancer, a decade after the first publication (Hanahan, 2011). One of the additions to these hallmarks is the reprogramming of energy metabolism of cancer cells. Cancer metabolism is an emerging theme in cancer biology. The discovery of oncogenes that regulate cell metabolism has stimulated research in tumorigenesis and the metabolic behavior of cancer cells (Cairns, 2011a). Oncogenes are involved in the proliferative signaling transduction of cells (Cantley, 1991) and, when mutated, can cause metabolic transformations. These metabolic transformations are intricately linked to cancer progression (Muñoz-Pinedo, 2012). Cancer cells have the capacity to shift their energy metabolism, so they grow faster and outcompete surrounding cells. Besides, some metabolic transformations affect the metastasis process of cancer cells in multiple ways. Firstly, correlations between metabolic behavior and metastatic target tissues has been found (Dupuy, 2015). Secondly, oncogenes were described that promote metastasis of cancer cells via the epithelial-mesenchymal transduction (EMT) (Konno, 2015).

Taken together, cancer metabolism is a rapidly emerging and promising theme in cancer research. The transformations cancer cells undergo could provide new targets for treatments, due to different metabolic behavior in comparison to non-tumorous cells. In this thesis an overview of the development of cancer metabolism will be provided, as well as the metabolic involvement in metastasis. Eventually, the influence of metabolic behavior on the mortality of cancer will be discussed. The leading question in this thesis is how transformations in cancer metabolism contribute to metastasis and metastasis induced mortality of cancer.

History of Cancer Metabolism

In the 19th century, major discoveries increased the interest in the research of metabolic activity of cells. Louis Pasteur discovered the fermentation process and Claude Bernard observed the conversion of glucose to lactic acid (Koppenol, 2011). These findings laid the foundation for metabolism research in cancer cells. This research mainly focused on the lipolysis and proteolysis of cancer cells, which were considered necessary to gain sufficient energy to multiply. The German scientist Otto Warburg was

one of the first to start investigating the glycolysis (Koppenol, 2011). He discovered that *in vitro* cancer cells have an upregulated lactic glycolysis in an oxygen rich environment. The studied cancer cells extruded more lactate than non-tumorous cells, which implied more active glycolysis. The energy metabolism of these cells has shifted to aerobic glycolysis and show embryonic glycolytic activity (Ferreira, 2012). This phenomenon is now known as the Warburg effect (Fig. 1). This was complementary to the findings of Louis Pasteur, who stated that oxygen inhibits lactic fermentation (Ferreira, 2012). Nevertheless, Warburg kept collecting evidence that his findings were true.

In subsequent years Warburg and others showed higher glucose uptake and lactate extrusion in *in vivo* tumor tissue (Ferreira, 2012). In his attempts to prove his right, Warburg discovered that the shift to aerobic fermentation was irreversible and probably transmitted after cell replication. This 'accidental' found phenomenon had major impact in the field of cancer metabolism. Warburg continued his work for decades after the discovery of the Warburg effect. In the 1950s he confirmed fermentation of purified cancer cells in fully aerated conditions (Ferreira, 2012). Warburg proposed that irreversible damage of respiration is involved in oncogenesis. The shift in energy metabolism to fermentation should be the leading cause for somatic cells to differentiate into cancer cells. This theory became known as the Warburg hypothesis. More precisely, the Warburg hypothesis states that damaged mitochondria are the driver of tumorigenesis because of the impairment of aerobic respiration in these cells (Koppenol, 2011; Ferreira, 2012; Vander Heiden, 2009).

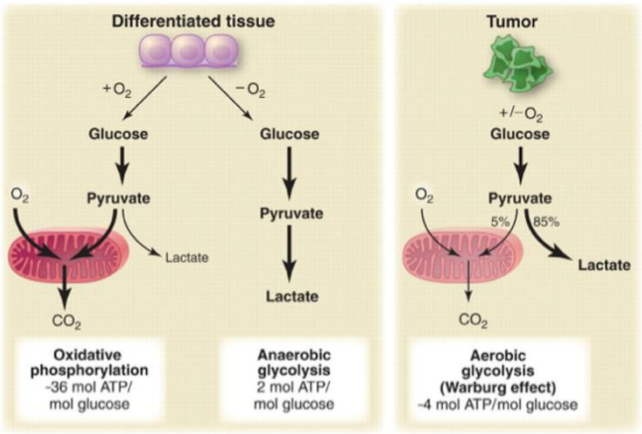

Fig. 1. Energy production pathways in differentiated and tumorous tissue. In normal differentiated tissue, the prominent energy pathway is activated according to the presence or absence of oxygen. In tumor tissue glycolysis is upregulated and predominant in both aerobic and anaerobic conditions. Adopted from Vander Heiden, 2009.

Although work performed by Warburg seems convincing, it was initially not acknowledged by the majority of cancer researchers. Especially the hypothesis that damaged respiration causes tumorigenesis became a frequently discussed topic among metabolism researchers. One opponent, Sidney Weinhouse, claimed that the Warburg hypothesis was false due to the fact that oxygen consumption was not impaired in cancer cells (Weinhouse, 1956). Instead of a change in energy metabolism, the cancer cells were only overactive energy producing cells. Weinhouse thought that the anaerobic glycolysis is so abundant in cancer cells, that the normal respiration and the normal Pasteur effect are incapable of eliminating it. In addition, Weinhouse and other scientist found oxidative phosphorylation was enhanced in cancer cells (Weinhouse, 1956). Besides, Weinhouse states that Warburg's results were misleading due to the generalization of respiratory and glycolytic activity of cells. Use of oxygen and energy production differ a lot between different cell types, even within tissues (Weinhouse, 1956).

Up to the 1970s cancer metabolism gained slightly more interest, but great breakthroughs remained scarce. However, 1976 became an important year for cancer metabolism research and had major influence on the future of the cancer research in general. Twenty years after his comments on Warburg, Weinhouse found no impairments of mitochondria in cancer cells, implying that damaged mitochondria could not be the cause of impaired aerobic respiration (Vander Heiden, 2009). This suggests that there must be a different explanation for aerobic glycolysis than originally postulated by Warburg. 1976 was also the year that the first oncogenes were described (Ferreira 2012). The

discovery of oncogenes shifted the focus mainly to the genetic aspects of cancer and reduced the interest in cancer metabolism even more. Cancer research, however, made significant progress in the following decades. Knudson described the first tumor-suppressor gene and links between cell proliferation and oncogenes and tumor-suppressor genes were found (Ferreira, 2012). Even though Warburg found that cancer cell transformations were transmitted after cell replication, no direct connection between cancer metabolism, oncogenes and tumor-suppressor genes was made. Cancer became known as a stepwise genetic disease caused by abnormally activated oncogenes and tumor-suppressor genes, whereby abnormalities are preserved during proliferation (Ferreira, 2012). The hallmarks of cancer were established after the turn of the century (Hanahan, 2000). The hallmarks are a set of cellular transformations a cell must undergo to be able to become oncogenic.

The real rejuvenation of interest in cancer metabolism began only few years ago. It started when the connection between oncogenes and metabolic processes became clear (Koppenhol, 2011). Oncogenes are often involved in proliferation and, when damaged, may contribute to the metabolic changes during tumorigenesis (Muñoz-Pinedo, 2012). Regarding Warburg's research in the 1920s, we now understand that the ability of cancer cells to shift their energy metabolism allows them to generate all components required for sustained cellular proliferation (Bates, 2015). All successful cancers switch to glycolysis; many of the mutations that drive proliferation also alter the metabolic regulation of these cells. Whether the Warburg effect is a cause or consequence of cancer is still under debate (Ferreira, 2012). Indisputably, cancer metabolism is now regarded as a hallmark of cancer (Hanahan, 2011) and more and more information about the metabolic involvement in cancer is currently gathered. Since cancer metabolism is regarded as a hallmark of cancer, metabolic transformations are though of to be necessity for tumorigenesis. Whether there are particular metabolic transformations that are present in different tumorigenesis processes is rather unclear.

Metabolic Transformations in Cancer Cells

Cancer is very difficult to cure, mostly due to the heterogeneity. Despite all the research and drug development that has been done in the past fifty years, no major breakthrough seems to be the absolute cure for cancer (Pedersen, 2007). Many cancer treatments are based on heavy anti-proliferative medication or radiation, which cause a lot of unwanted damage to side tissue. The problems with cancer treatments include the application and the nonspecific targeting which make it very hard to control. New antimetabolite chemotherapies frequently focus on a single abnormality in the cell, like enhanced protein expression (vander Heiden, 2011). Cancer is a multifactorial disease in which several genes are mutated at the same time. Focusing on one target at the time is often not enough to completely eliminate the cancerous tissue (vander Heiden, 2011).

Alternately, metabolic interventions in cancer cells are a promising new target because metabolic behavior in cancer cells differs from normal cells. Instead of using interventions based on a specific cancer cell marker, therapies addressed to a physiological state of a cancer cell could be developed (Ferreira, 2012). To generate those kind of treatments a lot of knowledge about specific metabolic transformations in developing cancer cells is required. Nowadays we know much more about the metabolic behavior of cancer cells. However, to determine what allows oncogenic cells to optimize their propagation we need to further investigate the origin of the mutability of cancer cells. Cells become oncogenic when suffering from DNA damage, more specifically due to mutations in oncogenes or tumor-suppressor genes. Oncogenes and tumor-suppressor genes are bioenergetic arbiters involved in the metabolic behavior of cells (Ferreira, 2012). An overview of oncogenes and tumor-suppressor genes directly involved in the metabolic pathways in proliferating cells is provided in Fig. 2.

Many oncogenes are tyrosine kinases which are associated with cell proliferation via regulation of glucose metabolism (vander Heiden, 2009). When exposed to growth factors tyrosine kinases inhibit the late steps of glycolysis, enabling the cell to use glycolytic intermediates for biosynthesis and NADPH production. When mutated, tyrosine kinases gain function and upregulate the production of macromolecules necessary for proliferation. One protein that is directly regulated by tyrosine kinases is pyruvate kinase M2 (PKM2) (vander Heiden, 2009). PKM2 is usually only present in proliferating cells. When phosphorylated by tyrosine kinases, PKM2 gets into a low-activity state. This low-activity state constitutes a molecular switch that allows cells to use glycolysis for energy production instead of the citric acid cycle and oxidative phosphorylation (vander Heiden, 2009). Oncogenic cells are associated with low-active PKM2, giving the cell proliferation characteristics (Muñoz-Pinedo, 2012). These characteristics involve biosynthesis of nucleotides and growth stimulation even if there are no growth factors engaged.

As exemplified by tyrosine kinases, growth factors have very important regulatory functions in proliferating cells and are related to cancer evolvement. Many oncogenes are stimulated by growth factors and, when mutated, frequently lose their sensitivity to growth factor withdrawal (Muñoz-Pinedo, 2012). One of the key metabolic regulators of proliferating cells activated by growth factors is the Myc oncogene. Myc is an iconic oncogene of which the metabolic changes driven by mutations in its gene precede tumor formation. (Hu, 2011). This implies importance of Myc mutations in the developing cancer cell to become a tumor. Myc activates thousands of genes involved in cellular

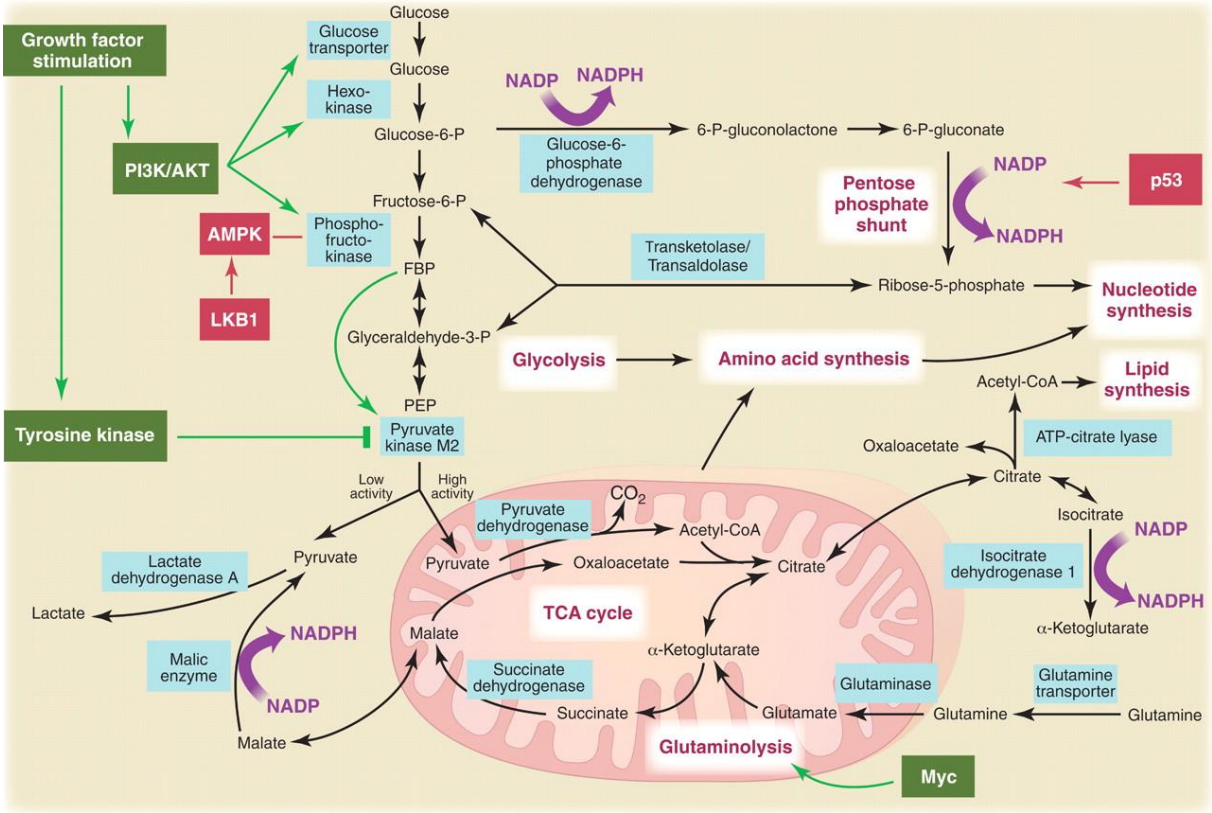

Fig. 2. Metabolic regulation pathways in replicating cells. The effects on glycolysis of PI3K and PKM2 in response to growth factors can be found in the upper left. The citric acid cycle (TCA cycle; inside mitochondrion) is mostly regulated by the Myc oncogene and the efflux of glycolysis. Biosynthesis of macromolecules (right; nucleotide/lipid synthesis) is fueled by all energy producing pathways and regulated by many genes (e.g. Myc, p53). In the green boxes regulatory oncogenes are displayed, in the red boxes tumor-suppressor genes. Critical enzymes are labeled in blue. Inside the white boxes, hallmark processes are shown. Adopted from Vander Heiden et al., 2009.

proliferation (Muñoz-Pinedo, 2012). First of all, Myc has major impact on the glutamine metabolism in proliferating cells. Glutamine is a major resource required for biosynthesis and anabolic processes in cancer cells (Gao, 2009). Many genes involved in the glutamine metabolism are directly or indirectly regulated by Myc (vander Heiden, 2009). In glutaminolysis, Myc upregulates enzymes by repressing the expression of inhibiting microRNAs (Cairns, 2011b). In particular, interactions between Myc and microRNAs are involved in the regulatory mechanism of glutamine metabolism in human cancer (Gao, 2009). The Myc oncoprotein is generally known to regulate microRNAs and enhance proliferation (Gao, 2009). Next to glutamine metabolism, upregulation of Myc contributes to the Warburg effect by directly enhancing aerobic glycolysis and by stimulating the expression of the before mentioned PKM2 (Muñoz-Pinedo, 2012). Myc regulates alternative splicing that generates the PKM2 isoform under rapid growth conditions (Cairns, 2015). Both glutamine metabolism and glycolysis, where multiple pathways controlled by Myc are involved, contribute to the proliferating biosynthesis.

Another pathway that is activated by growth factors is the signaling pathway of the oncogene phosphoinositide 3-kinase (PI3K). The PI3K signaling pathway is one of the major factors regulating the glucose uptake and glycolytic metabolism, mostly by inducing the flux of the early steps of glycolysis (vander Heiden, 2009; Muñoz-Pinedo, 2012). PI3K stimulated biosynthesis of lipids as well (Muñoz-Pinedo, 2012). In cancer cells, upregulated PI3K enhances proliferation independently of growth factors (vander Heiden, 2009). Human cancer cells often become dependent on PI3K signaling or the downstream signaling molecules (vander Heiden, 2009). Growing evidence suggests that cancer cells with overactive PI3K signaling depend on glycolysis.

Besides oncogenes, which gain an oncogenic function when mutated, there are proteins that try to protect the cell from developing cancer. These proteins become oncogenic when their genes are mutated or deleted and a loss of function appears. The most important so called tumor-suppressor gene is p53. p53 is a protein that is seen as the guardian of the genome and is mutated in over half the cancer cases (Muñoz-Pinedo, 2012). The protein regulates many genes involved in apoptosis, cell cycle and DNA repair among others. Mutations in the TP53 gene, which encodes p53, result in the loss of function of p53; non-functional p53 is a common feature in the majority of cancers (Muller, 2013). One aspect of loss of p53 function is induction of the Warburg effect through dysregulation of the electron transport chain (Muñoz-Pinedo, 2012). p53 is activated when cells undergo metabolic or hypoxic stress (Muñoz-Pinedo, 2012). In normal conditions, p53 blocks the pentose phosphate pathway (called pentose phosphate shunt in Fig. 2) and thereby suppresses glucose consumption and biosynthesis of nucleotides among others (Jiang, 2011). p53 protects cancer development in multiple ways, but it loses all its protective functions when mutated. Besides losing protective functions, mutant p53 also facilitates activation of nucleotide biosynthesis genes (Pavlova, 2016).

Increasing knowledge about the genetic background rejuvenated the interest on the Warburg effect as well. Generally, researchers attempted to elucidate the underlying regulation of the Warburg effect. One explanation, which is now considered to be true, was already proposed by Warburg himself. The metabolic shift to mainly glycolysis is beneficial for the oxygen distribution in a solid tumor. Oxygen levels in a tumor can drop to near zero and tumor cells are required to use glycolysis for energy production (Vaupel, 2004; Muñoz-Pinedo, 2012). Prior to the angiogenesis, cells with high glycolic activity have higher survival chances and outcompete other cells (Cairns, 2015). Because most cancer cells originate for the same cell, and metabolic regulation is transmitted through cell replication, many cells show similar metabolic behavior. However, in ecologic perspective using a less efficient energy production pathway seems paradoxical (Cairns, 2015). Studies on energy distribution indicates that ATP levels may never be the limiting factor in proliferating cells (vander Heiden, 2009). These data drove researchers to investigate beyond the scope of bioenergetics (Cairns, 2015). Nowadays, the most

prominent explanation for the Warburg effect is the benefits glycolysis has over oxidative phosphorylation. In glycolysis, not only energy is produced but also molecular building blocks necessary for replication (vander Heiden, 2009). These building blocks include amino acids, nucleotides and lipids that are essential for rapid replication within tumors. Unfortunately for these cells, the energy production is way lower when shifted to glycolysis. However, cells can permit to produce energy in a less efficient way because resources are not scarce in proliferating mammalian cells (vander Heiden, 2009).

The Warburg effect is closely related to proteins called Hypoxia-Inducible Factors (HIFs). HIFs are transcription factors that are stabilized in the hypoxic conditions in tumors, but can be stimulated by inactivating mutations of tumor-suppressor genes as well (Muñoz-Pinedo, 2012). Enhanced activity of HIFs in cancer cells frequently occur during cancer progression (Mimeault, 2013). This enhanced activity may result in the upregulation of stem cell gene products and survival signaling elements. The altered gene products are associated with pluripotency, EMT and the shift to aerobic glycolysis (Mimeault, 2013). In other words, upregulated HIF activity leads to crucial alterations for cells to generate cancer-like characteristics and enable these cells to reproduce. Besides higher glycolytic activity, HIFs regulate downstream effectors that reduce the flow of pyruvate used in the citric acid cycle; thereby reducing oxidative phosphorylation and increasing biosynthesis (Muñoz-Pinedo, 2012). HIFs can also contribute to the transport of lactate out of the cell (Mimeault, 2013). This enables the cell to survive, which otherwise would die due to lactate overproduction. The increasing acidity due to lactate accumulation and extrusion promotes tumor cell adaptation and may evolve the tumor cell niche (Cairns, 2011a). In addition, extracellular lactate accumulation and raised acidity favor normal cell death and degradation of the extracellular matrix (Mimeault, 2013). All of these transformations are essential for cancer cells to perform metastasis. When cancer cells start to metastasize, disease development and mortality rise incredibly.

Metabolic Regulation of Metastasis in Cancer Cells

That cancer cells can metastasize has been known for more than 100 years, but only recently more insights about the mechanisms behind metastatic tumor formation were obtained (Fidler, 2003). Already in 1889, Stephen Paget suggested that metastasizing cells do not home in random tissues but have specific affinity for specific types of tissue (Fidler, 2003). This phenomenon is called the 'seed and soil' hypothesis. Although the hypothesis was challenged, it still holds up nowadays.

Metabolic transformations and metastasis are considered to be a hallmarks of cancer and their interaction has been studied intensively in recent years. Nevertheless, not much is known about metabolic alterations that accompany tumor metastasis (Dupuy, 2015). Just like all other hallmarks of cancer, cancer metabolism and metastasis are under regulation of many molecular mechanisms (Cairns, 2015). Growing evidence indicates that metabolic regulation is key in metastasis. Tumor cell adaptations in response to hypoxic stress allows the cells to overcome nutritive deprivation and enables them to metastasize (Vaupel, 2004). Moreover, to recall to the previous section, HIFs are responsible for many transformations that enables the cells to become metastatic (Mimeault, 2013). The induction of stem cell-like metabolic characteristic in combination with stimulation of gene products that cause EMT, invasion and metastasis are crucial elements for cancer cells to efficiently spread to other tissues. Besides HIFs, mutated Myc proteins also contribute to metastasis by promoting proliferation, cell survival, EMT and blockage of differentiation (Wolfer, 2010).

In metastasis, some general mechanisms of dissemination exist, which enable tumor cells to abandon their primary environment, but specialized mechanisms are required to be able to infiltrate other

tissues (Nguyen, 2009). One of the general mechanisms involved in dissemination is inflammation of the tumor environment (Coussens, 2002). Inflammation of the tumor cell environment regulate growth and differentiation of cells, and promotes migration by releasing survival factors, promoting angiogenesis and remodeling the extracellular matrix (Coussens, 2002). When metastasizing, metabolic regulators of the cell may determine where the cancer cells can home (Dupuy, 2015). Recent studies indicate that heterogeneity in tumors plays an important role in metastatic behavior of cancer cells. First of all, the expression of genes regulating proliferation, angiogenesis, cohesion, motility and invasion differ greatly between cells in the same tumor (Fidley, 2003). In tumor development, heterogeneity must be taken into account when looking at metastatic adaptation. Like initial cancer cells have their own features, homing tissues have specific characteristics as well and metastasized cells can only develop in specific organs (Fidley, 2003). Environmental elements are unique and the cells homing for a different type of tissue need to adjust to that. Accordingly, Dupuy et al. showed that metastasizing breast cancer cells from a heterogenic tumor home to different tissues according to different metabolic behavior (Fig. 3) (Dupuy, 2015). Cells that mostly use oxidative phosphorylation for energy production homed to the bone marrow and lungs, while cells using glycolysis nestled in the liver. After metabolic analysis of liver metastases, upregulated HIF activity was found which in its way activated PDK1. PDK1 is one of the before mentioned downstream effectors of HIFs that reduce the flow of pyruvate into the citric acid cycle (Muñoz-Pinedo, 2012). PDK1 blocks the conversion of pyruvate to acetyl-CoA and thereby drives alterations in metabolism. (Döppler, 2015). In breast cancer cells, the consequent metabolic shift regulates metastasis and the expression levels of PDK1 in the parental tumor cell determine to which tissue the cancer cells will metastasize (Döppler, 2015). High PDK1 activity is necessary to effectively metastasize to the liver, while low expression is required for affection of the lungs (Dupuy, 2015). Taken together, PDK1 is a major contributor to the metabolic reprogramming, and determines whether glycolysis or oxidative phosphorylation is predominant and thereby to which target tissue the cancer cells can metastasize.

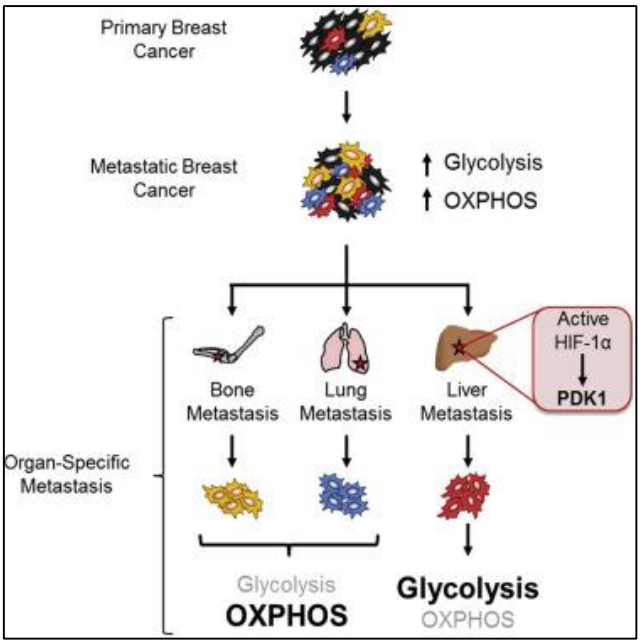

Fig. 3. Metabolic regulation and the consequent target tissues of the metastasized cancer cells. Cells relying mostly on oxidative phosphorylation metastasize to bone- and lung tissue, while cells performing glycolysis home to the liver. Adopted from Dupuy et al., 2015.

Although often related to developing cancer, metastasis is a very inefficient process in which only few of the migrating cells successfully home in a distant tissue (Mehlen, 2006). Nevertheless, it has been stated that metastasis is the cause of death in 90% of the human cancer cases (Mehlen, 2006). Yet the pathogenesis and the genetic and biochemical determinants of metastasis remain poorly understood (Mehlen, 2006; Chaffer, 2011). The most prominent way in which metastases are lethal is by accumulation of host tissue damage. This damage is a consequence of various pathological processes. A major pathological process that increases the risk of cancer mortality is cancer-associated cachexia. The malignancy of a tumor is often associated with the development of cachexia and metastasis (Pinto, 2015). Cachexia is an inflammatory condition associated with significant weight loss from fat and muscle mass and is apparent in 60 to 80% of patients with advanced cancer (Pinto, 2015). The involuntary loss of body mass contributes to progressive functional damage. The pathology of cachexia

is caused by variable combination of metabolic abnormalities and a protein and energy imbalance (Pinto, 2015). Nutritional treatment is not effective in cachexia patients and muscle mass cannot be maintained. In 1975, however, cachexia was reported as the major cause of death in only 1% of the cancer patients (Ambrus, 1975). Ambrus et al. stated that infection, respiratory failure, and hemorrhagic and thromboembolic phenomena were the most frequent causes of cancer mortality. Invasion of other tissues following metastasis should have been the cause of death in merely 10% of the fatal cancer cases (Ambrus, 1975). Nowadays, research indicates that more than 70% of the cancer patients die of the progression of underlying cancer, including metastatic disease (Khorana, 2007). However, this percentage is inconsistent in comparison to the before mentioned 90% of cancer fatalities due to solely metastatic disease. Cachexia, another progressive phenomenon, is responsible for 20% of cancer patient fatalities (Pinto, 2015). Second to cancer progression, the development of the thromboembolic phenomenon, which is responsible for the blockage of blood vessels, increases mortality 2,2 fold in cancer patients and this number is rising (Khorana, 2007). Infection has about the same mortality rates as the thromboembolic phenomenon, just under 10% of cancer patients (Khorana, 2007).

Different cancer types show different metastatic behavior. Some types of cancer metastasize to various tissue types; some others are able to colonize only a specific tissue. The other way around, there are tissues that are a frequent target for metastatic cells, such as lung, liver and bone, and tissues that are almost never harmed by metastatic cells (Percy, 1981). Breast cancer has the ability to home for the bone marrow, lung, brain and liver parenchyma, while prostate cancer can solely colonize the bone marrow (Nguyen, 2009). Perhaps accordingly, the five-year survival rate of breast cancer is lower than that of prostate cancer, respectively 89,7% and 98,9% (SEER Cancer Statistics Factsheets, 2016). Differences in mortality rates between cancer types is generally recognized and have been reported. For example, the five-year recurrence-free survival rate is 60 to 70% for stage I lung adenocarcinoma, while that rate is 98% for breast cancer in the same stage (Nguyen, 2009). Which role metabolic behavior has on metastasis and, eventually, mortality remains rather unclear. Although, very specific information has been gathered. An example would be that abnormal glucose metabolism is strongly associated with pancreatic cancer mortality (Gapstur, 2000). However, what has been clarified is that metabolic regulation is a major contributor to cancer development and metastasis, and that metabolic activity influences the metastatic behavior of the cancer cells. This interaction could be a promising target for future research in the search for new therapies to treat cancer.

Discussion

Through history, cancer metabolism has been an underestimated factor hallmark in the development of cancer and its pathology. It all began with the discovery by Otto Warburg of oxidative glycolysis in cancer cells, now known as the Warburg effect. Even though being doubted in the early years, the Warburg effect still is one of the main metabolic pathways in cancer. Many oncogenes and tumor-suppressor genes are involved in regulating metabolism in cancer cells, including the Warburg effect. These changes are important for cancer cells to become and develop cancer. In order of migratory behavior of cancer, hypoxia seems to be very important in the metastatic process. To become metastatic, cancer cells need to be able to leave their own environment and migrate to another one. HIFs and inflammation are important regulators in enabling cells to metastasize. Likewise, the Warburg effect helps oncogenic cells to grow and metastasize. What the exact influence of metastasis is in term of mortality remains uncertain, mostly due to inconsistencies in literature. Nevertheless, metastasis is a considerable cause of death in cancer, and the mortality can be induced in many different ways.

Taken together, it is very clear that metabolic shifts are a significant regulating factor in cancer and provide great new targets for treatment of this horrible group of diseases.

In terms of cancer therapy, since the rejuvenation of cancer metabolism multiple agents targeting metabolic enzymes have been developed (Galluzzi, 2013). Unfortunately, these agents target both malignant and rapidly proliferating cells. Agents targeting general proliferative mechanisms often have adverse side effects. For example, the currently used cancer therapeutic agent methotrexate inhibits metabolic biosynthesis of amino acids and nucleotides, but also has the adverse effect of causing inflammation in the gastrointestinal tract due to induction of rapid replicating cell death (Duncan, 2003; Dawson, 1965; vander Heiden, 2011). On the other hand, the shifted metabolic state of cancer cells could be a great new target for cancer interventions (vander Heiden, 2011). Apparently, the shift to aerobic glycolysis is a clear target for cancer therapies as proliferating cells do not show this behavior (vander Heiden, 2011). All of the oncogenes and tumor-suppressor genes mentioned in the metabolic regulation section have an influence on the glycolytic shift. There are even more genes involved in the process and glycolytic effector genes are not necessarily regulated by one single gene (Hu, 2011). In aerobic metabolism, specific proteins and enzymes are upregulated. PKM2 is the isoform of pyruvate kinase that is most prominent in tumors, promoting aerobic glycolysis and cell proliferation. Targeting PKM2 has shown to slow cell proliferation in cell culture (vander Heiden, 2011). This implies that targeting PKM2 could slow down tumor formation by downregulating glycolysis. As earlier described, an interesting finding is that overexpression of glycolytic energy production, which is way less efficient than oxidative phosphorylation, can be sustained due to the fact that resources never are scarce in proliferating cells. This implies that cells do not have any difficulty in regulating their energy metabolism, perhaps by overfeeding. Caloric restriction, which is the reduction of calorie intake without malnutrition, has been suggested as a cancer prevention therapy in primate species (Colman, 2009). Recent data shows that sustained caloric restriction reduces cancer initiation, development and metastasis in mice (Lv, 2014). However, caloric restriction could perhaps be not applicable in patients that are already suffering from cancer because of the risk of cachexia.

Metabolic agents could be applied to intervene with cancer development and growth. Cancer cells use much more glucose than normal cells due to the Warburg effect. Therefore, the metabolism could be interrupted by using ineffective glucose molecules to deplete cancer cells of energy (Zhang, 2014). An inhibiting glucose analog, 2-Deoxy-D-glucose (2DG), is a potential potent antitumor agent that even in relatively low dosage is toxic to certain cancer cells. The therapeutic effect, unfortunately, of 2DG is limited in many cancer types, but in combination with other therapeutic agents or radiotherapy may exert an interactive therapeutic action (Zhang, 2014). In terms of altered lipid metabolism, a hallmark process in cancer metabolism (Fig 2.), abnormal choline metabolism is associated with oncogenesis and tumor progression (Glunde, 2011). Specific changes in choline phospholipid metabolism are associated with a more aggressive cancer phenotype (Glunde, 2006). Therapeutic interventions of these lipids reduced the aggressiveness of the cancer and reversed the metabolic alterations. Knocking down the choline phospholipid metabolism could be performed *in vivo* with siRNAs, among others (Glunde, 2006). Altogether, the field of cancer metabolism is emerging and therapeutic agents are under investigation. Cancer metabolic interventions have the potential to become a therapeutic application in cancer treatment.

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